

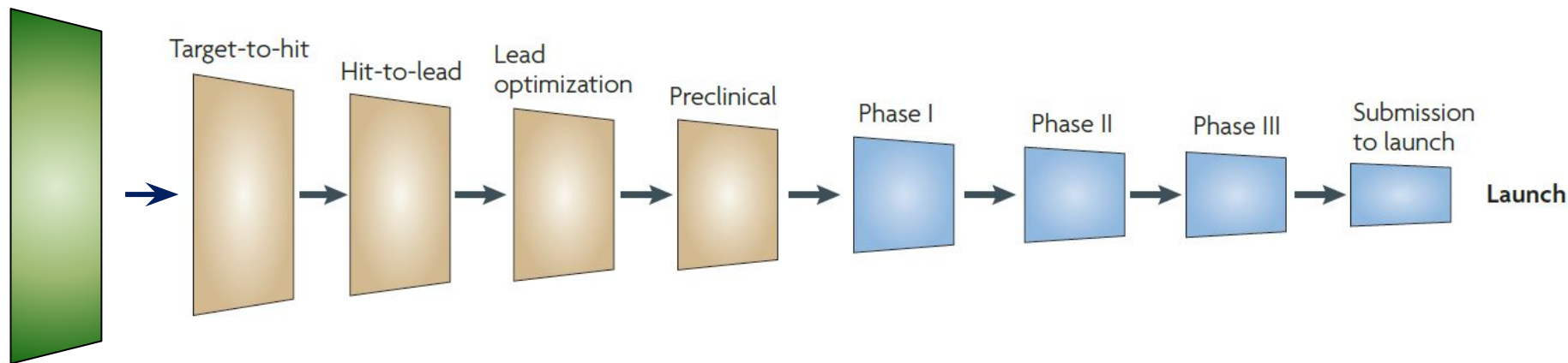
# What can we do if there are no good targets

*Mathematical and Computational Biology in Drug Discovery  
(MCBDD) Module II*

*Dr. Jitao David Zhang  
March-April 2021*

# The linear view of drug discovery builds on target-based approaches

Target identification & assessment

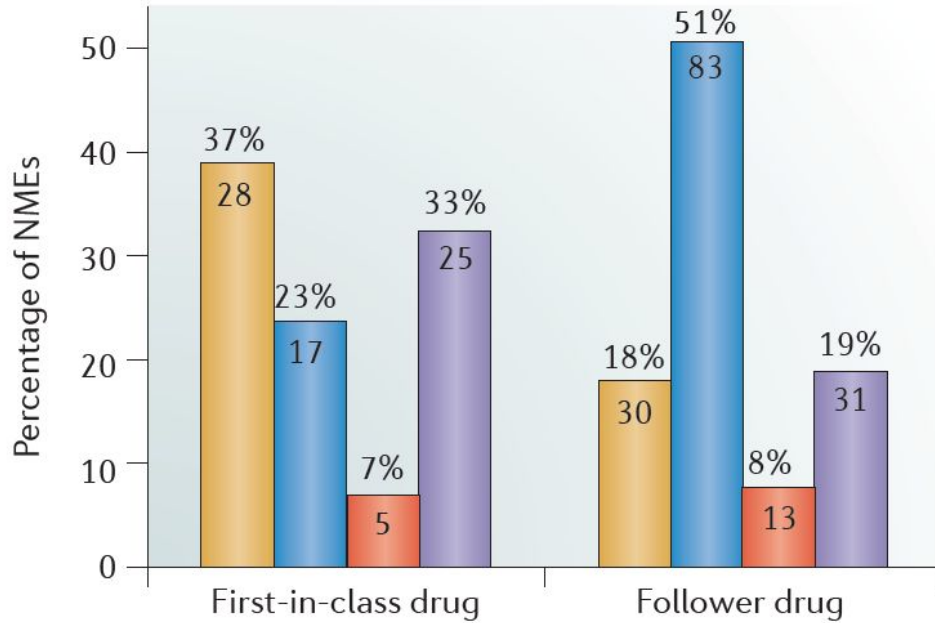
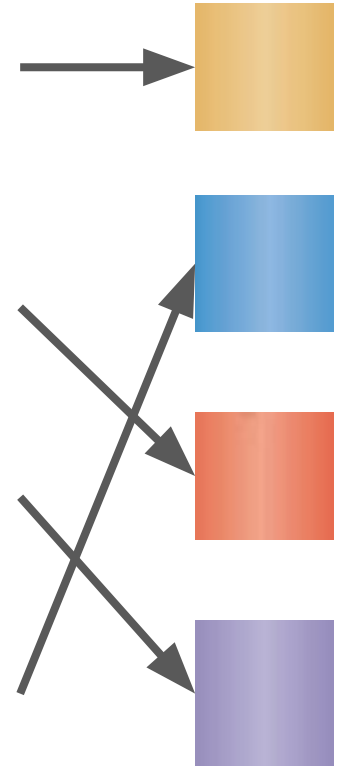


# Five strategies when no good target is found

1. Phenotypic drug discovery
2. Natural products
3. Biologics
4. Interaction-based (multispecific) drug discovery
5. Drug repurposing or combination studies

# Connect the lines!

- Phenotypic screening
- Modified natural products
- Biologics
- Target-based screening



# Phenotypic screenings by agent and readout

**Agent**

High-throughput screening  
libraries ( $\geq 10^6$  molecules)

Genetic libraries ( $\sim 10^4$ )

Natural products and chemo-  
genomic libraries ( $\sim 10^3$ )

Custom libraries ( $\sim 10^0$ - $10^2$ )

**Feasibility**

Reporters

Gene  
expression

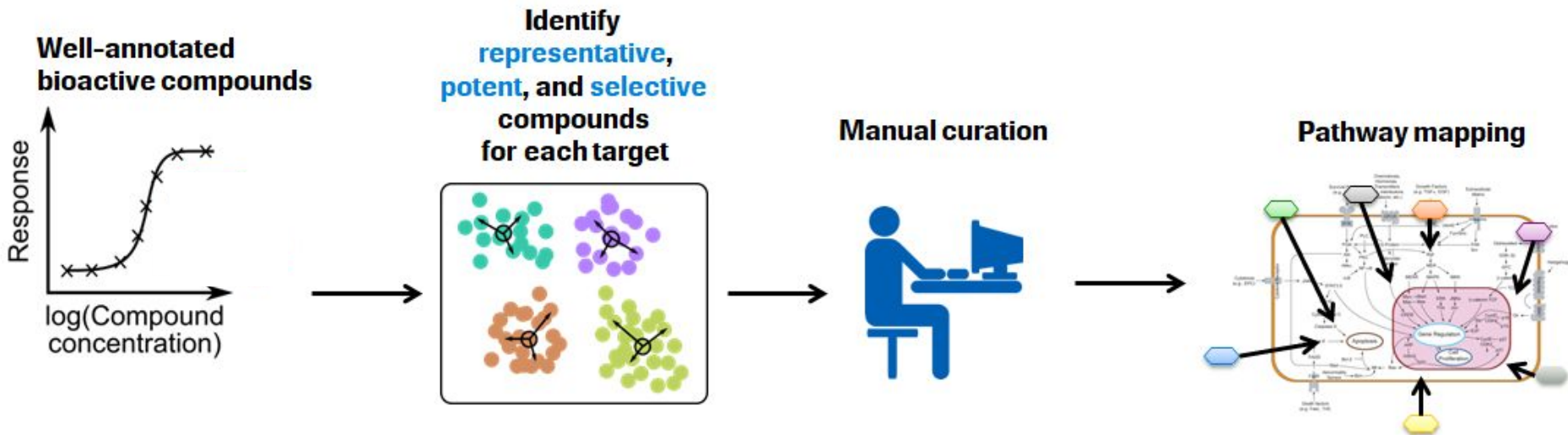
Cellular  
morphology

Organ/tissue  
phenotype

Organism  
phenotype

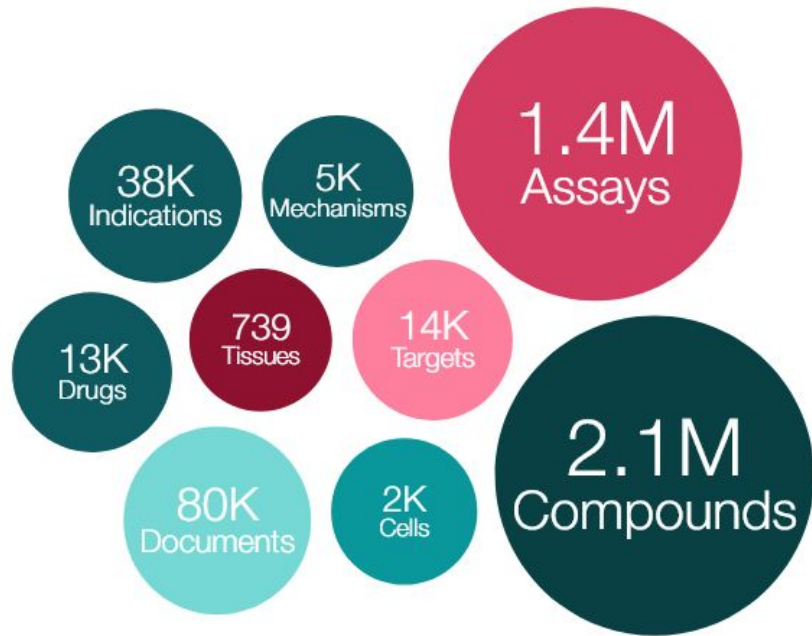
**Readout**

# The Small-molecule Pathway Research Kit (SPARK)



# The ChEMBL database

- An example of query: [aspirin](#).
- Systematic and programmatic accession via [ChEMBL API](#) ([source code](#)).
- We can use **dose-response data** to annotate the *triplets* of compound, assay activity, and targets.



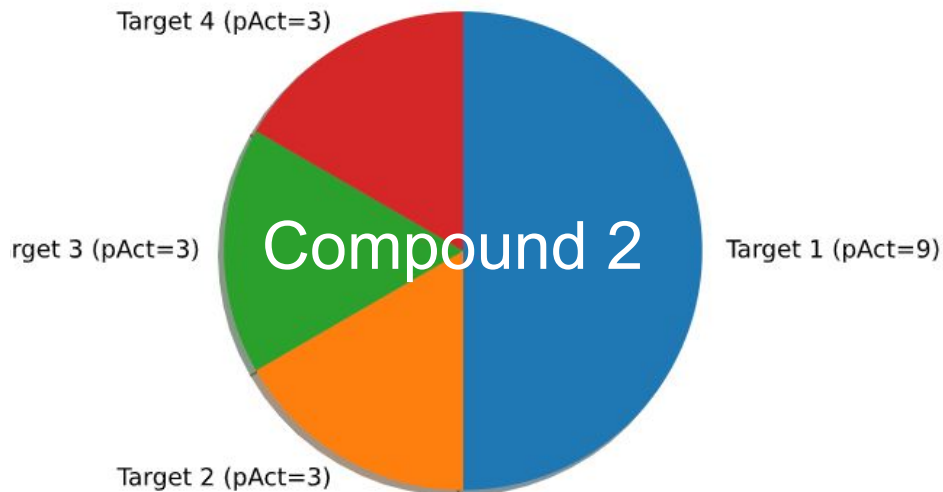
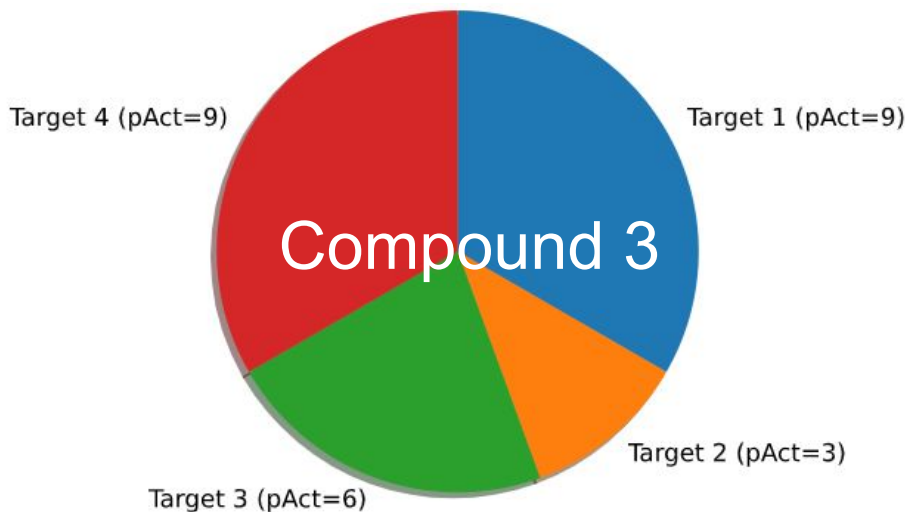
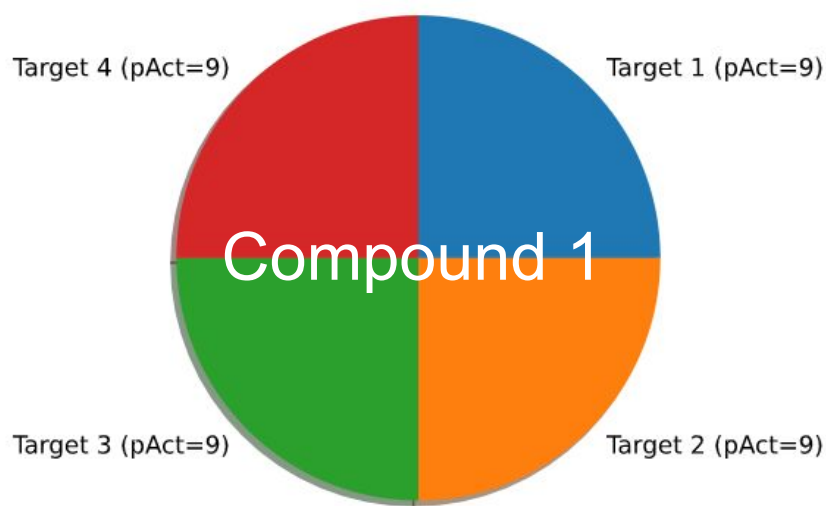
March 2021

# Discussion

1. Why do we care selecting  
*representative, potent, and selective*  
compounds for each target?
2. How to define following terms  
mathematically ...
  - a. Representativity?
  - b. Potency?
  - c. Selectivity?

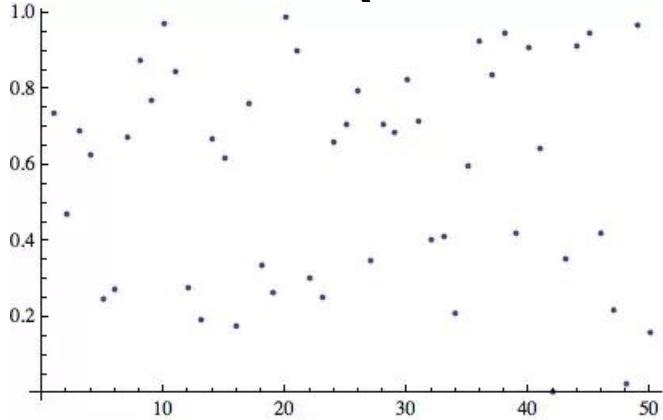


# A toy example about how to quantify a compound's potency and selectivity

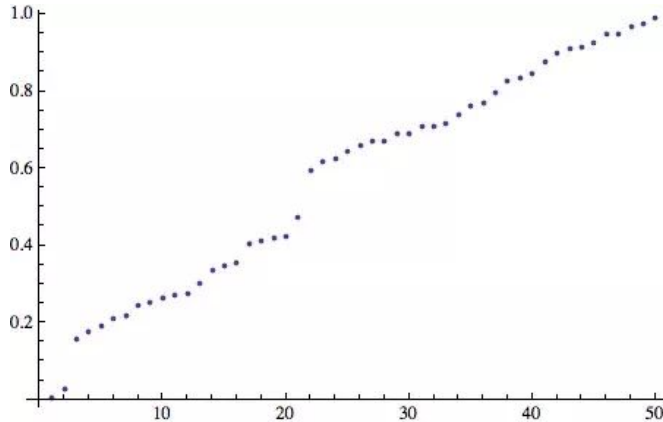


# The Gini Index (a.k.a. Gini Coefficient)

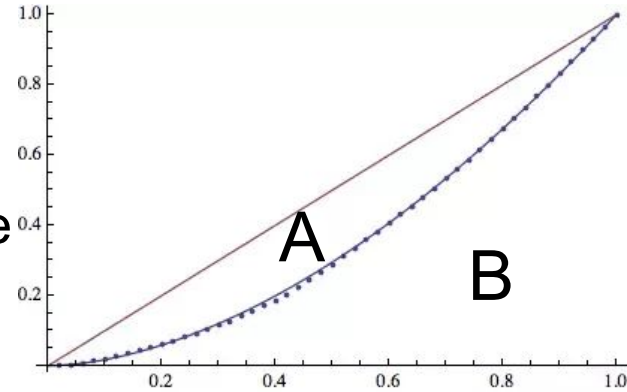
A random  
vector of  
50 values



Sorted  
from low  
to high



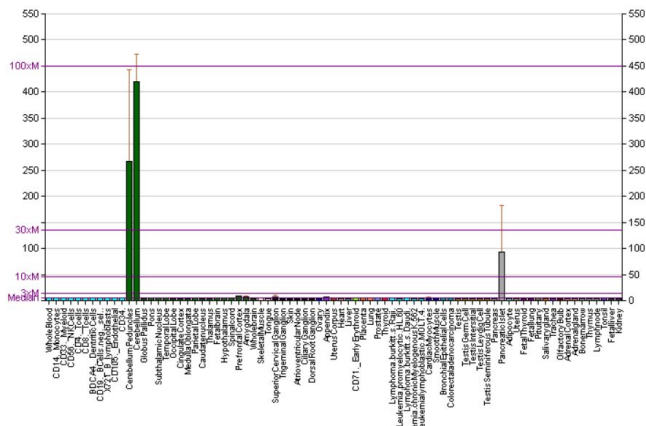
The Gini  
Index is  
calculated  
based on the  
cumulative  
distribution



$$G = A / (A + B)$$

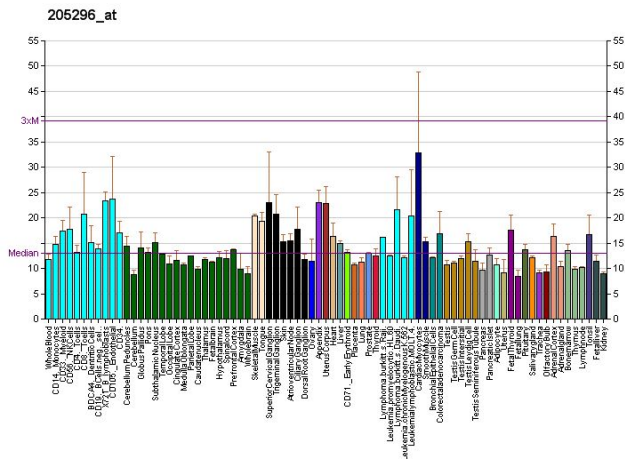
# The Gini Index quantifies inequality/ selectivity

*NEUROD1*

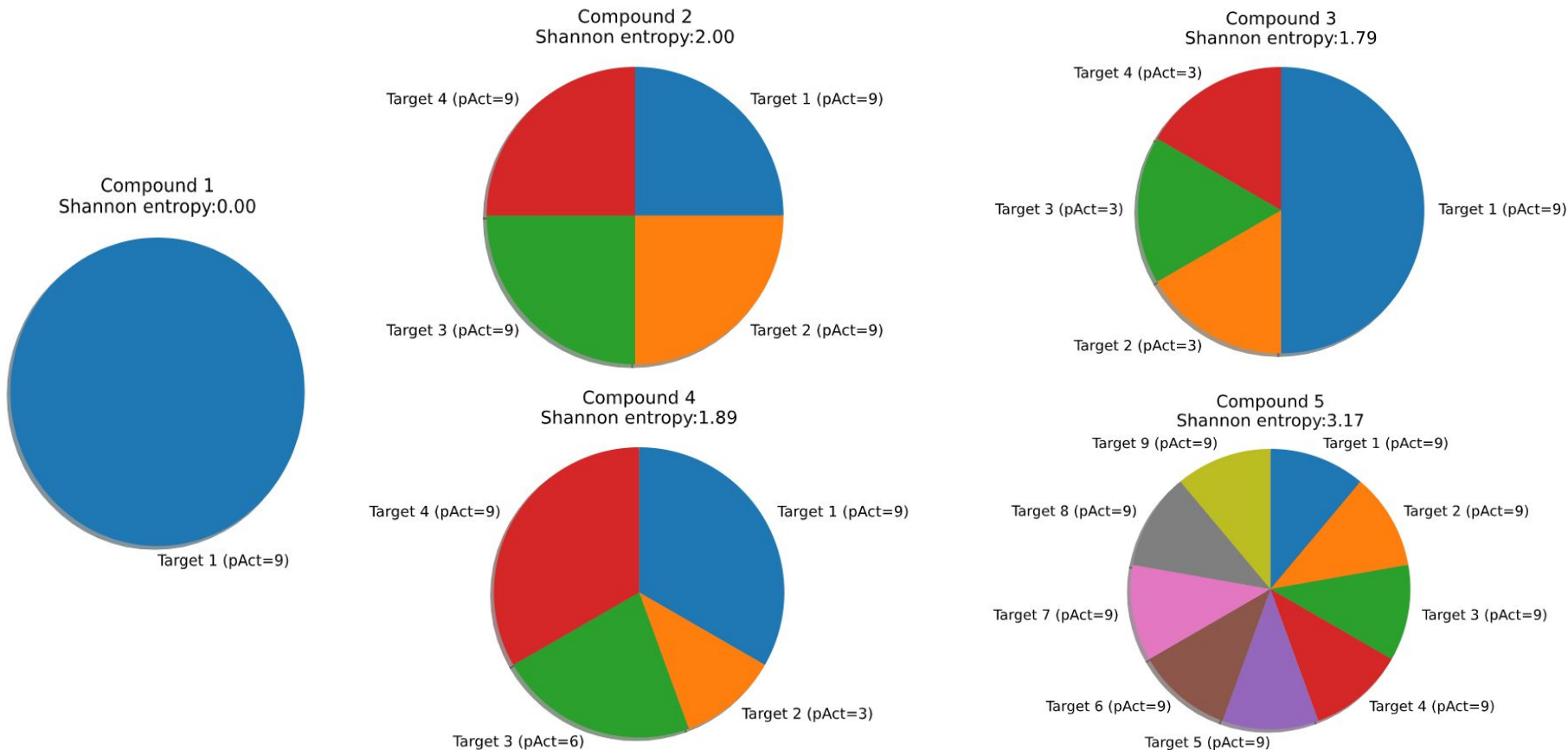


The Gini Index of expression of *NEUROD1* across tissues is near 1, whereas that of *RBL1* is near 0.

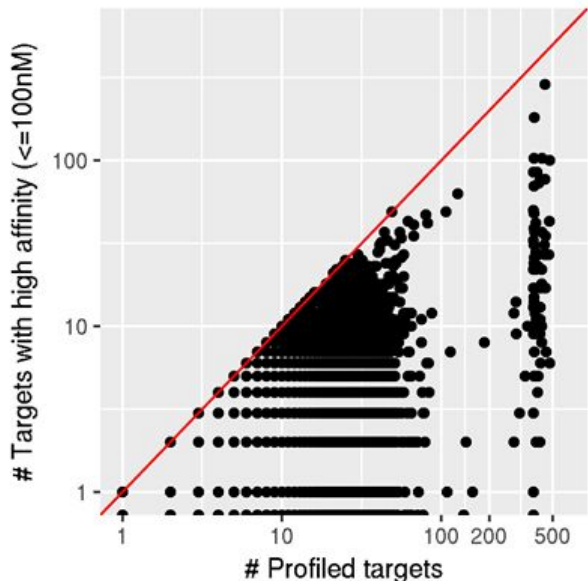
*RBL1*



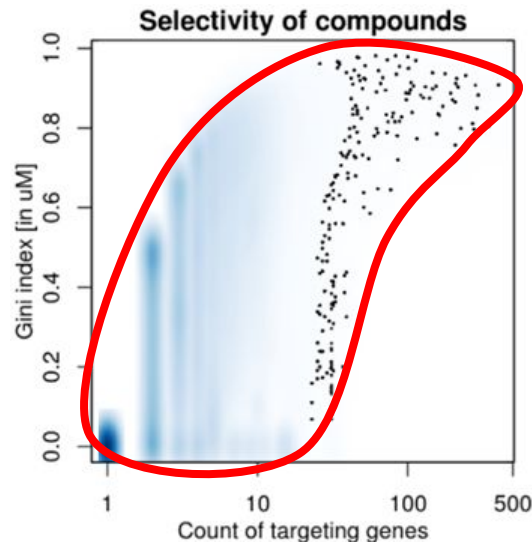
# An alternative metric: Shannon's Entropy



# Count of targets and selectivity of ChEMBL molecules

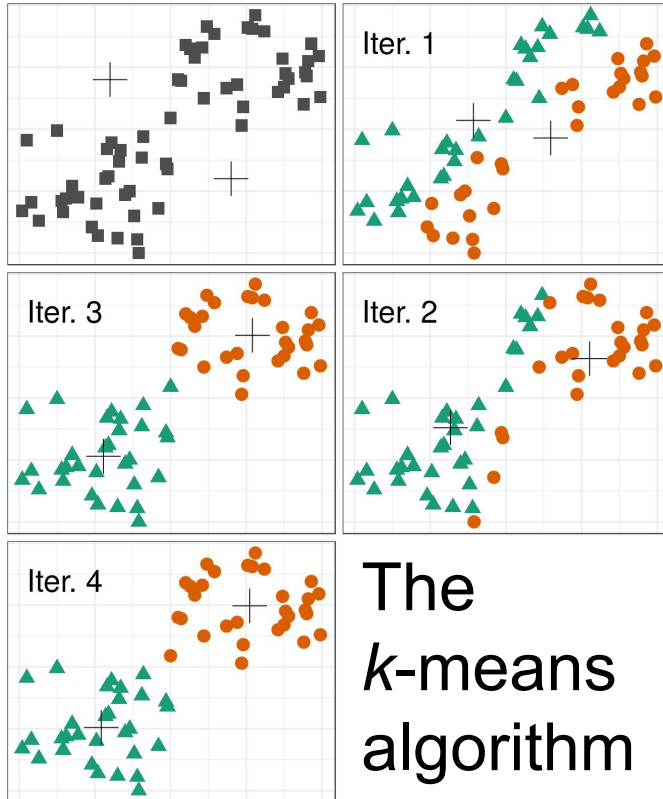


With some exceptions, most compounds are profiled against <100 targets. We distinguish between specific and pleiotropic compounds.



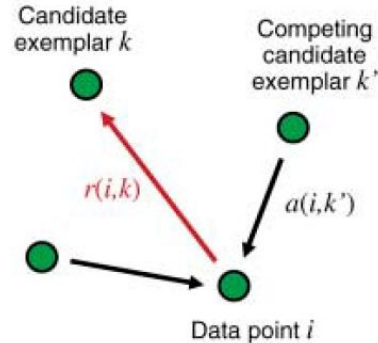
The **shark-fin shape** curve suggests that frequently profiled compounds tend to be more selective (and *vice versa*).

# Unsupervised clustering



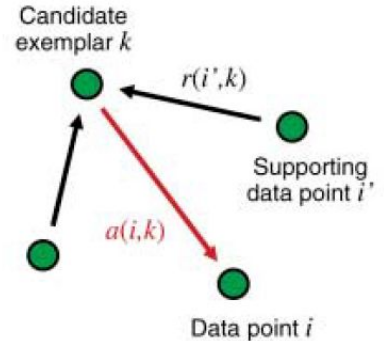
**B**

Sending responsibilities



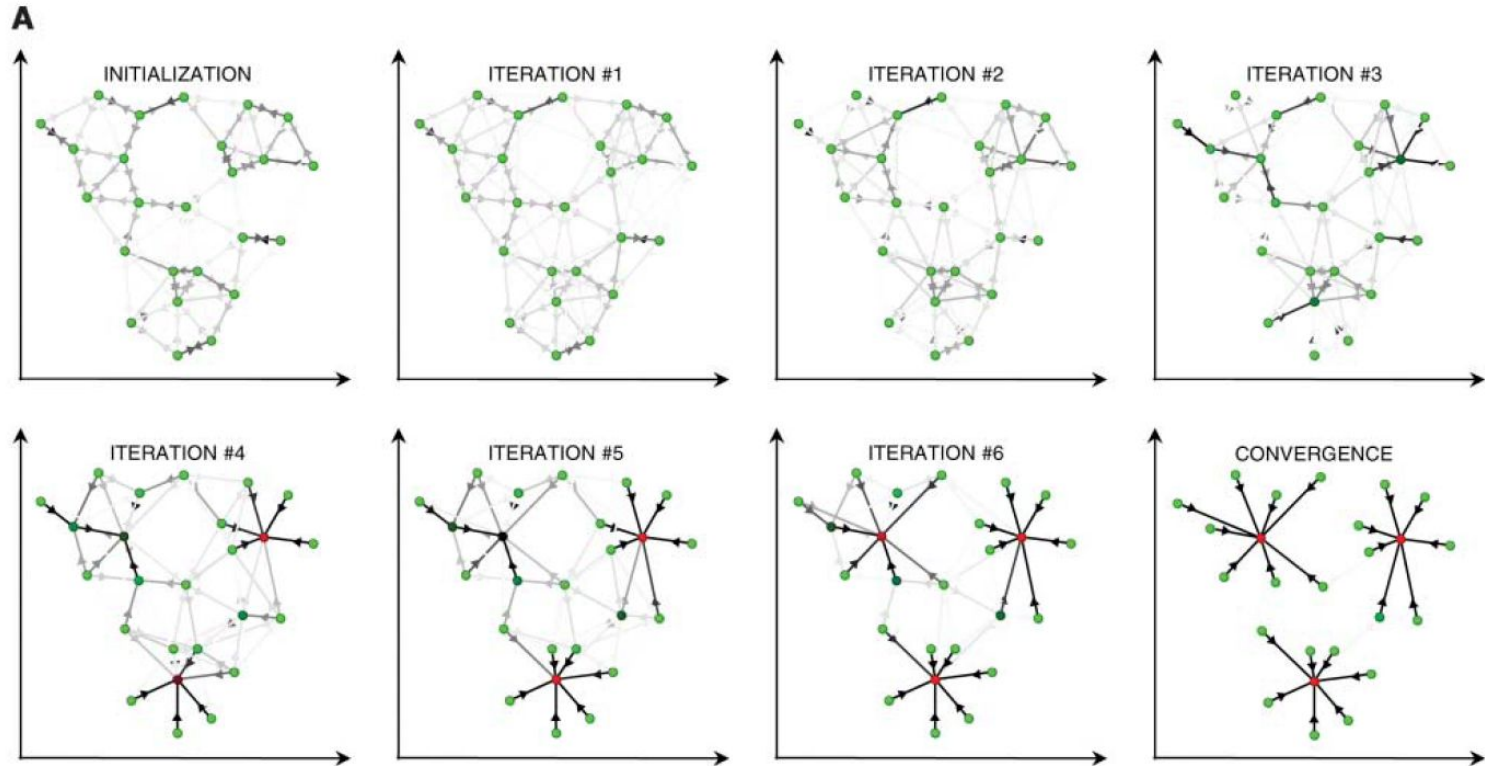
**C**

Sending availabilities



Affinity Propagation updates **responsibilities** and **availabilities** iteratively

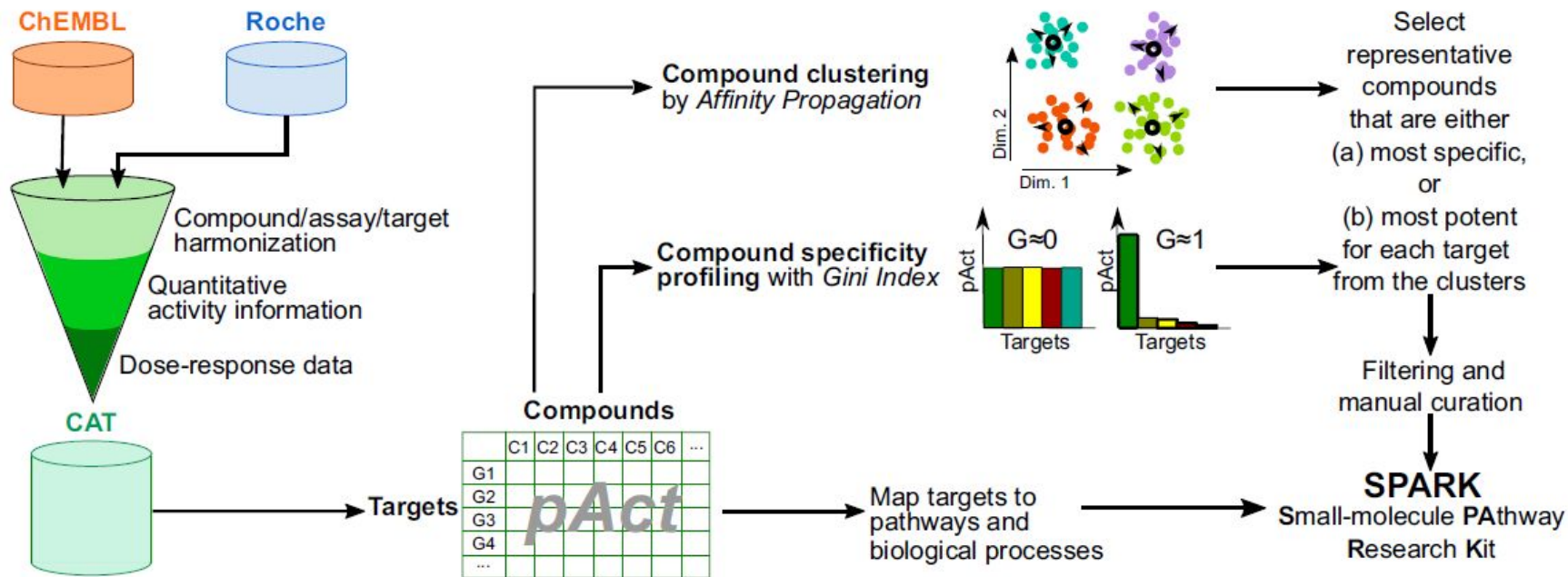
# Affinity Propagation in action



[A movie of iterations](#)



# Construction of SPARK in detail



## Harmonization

... of public and Roche internal data

## Machine learning

... to select compounds

## Pathways

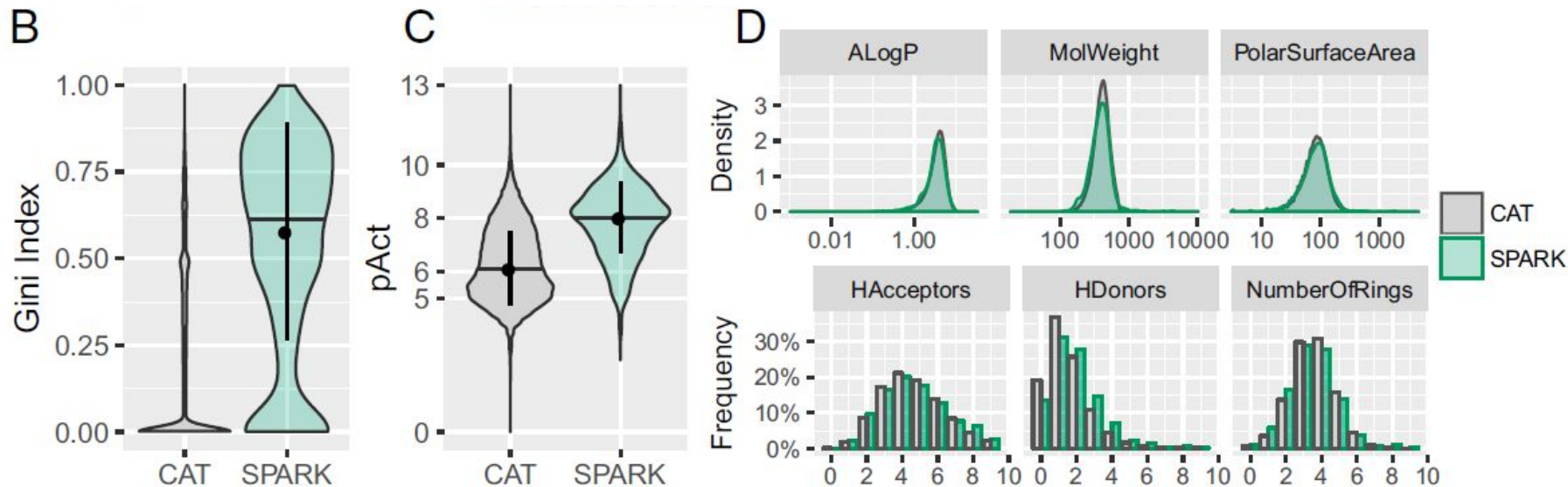
... mapped to compounds

## Curation

... to enrich quality compounds



# SPARK covers the chemical space evenly with representative, potent, and specific compounds

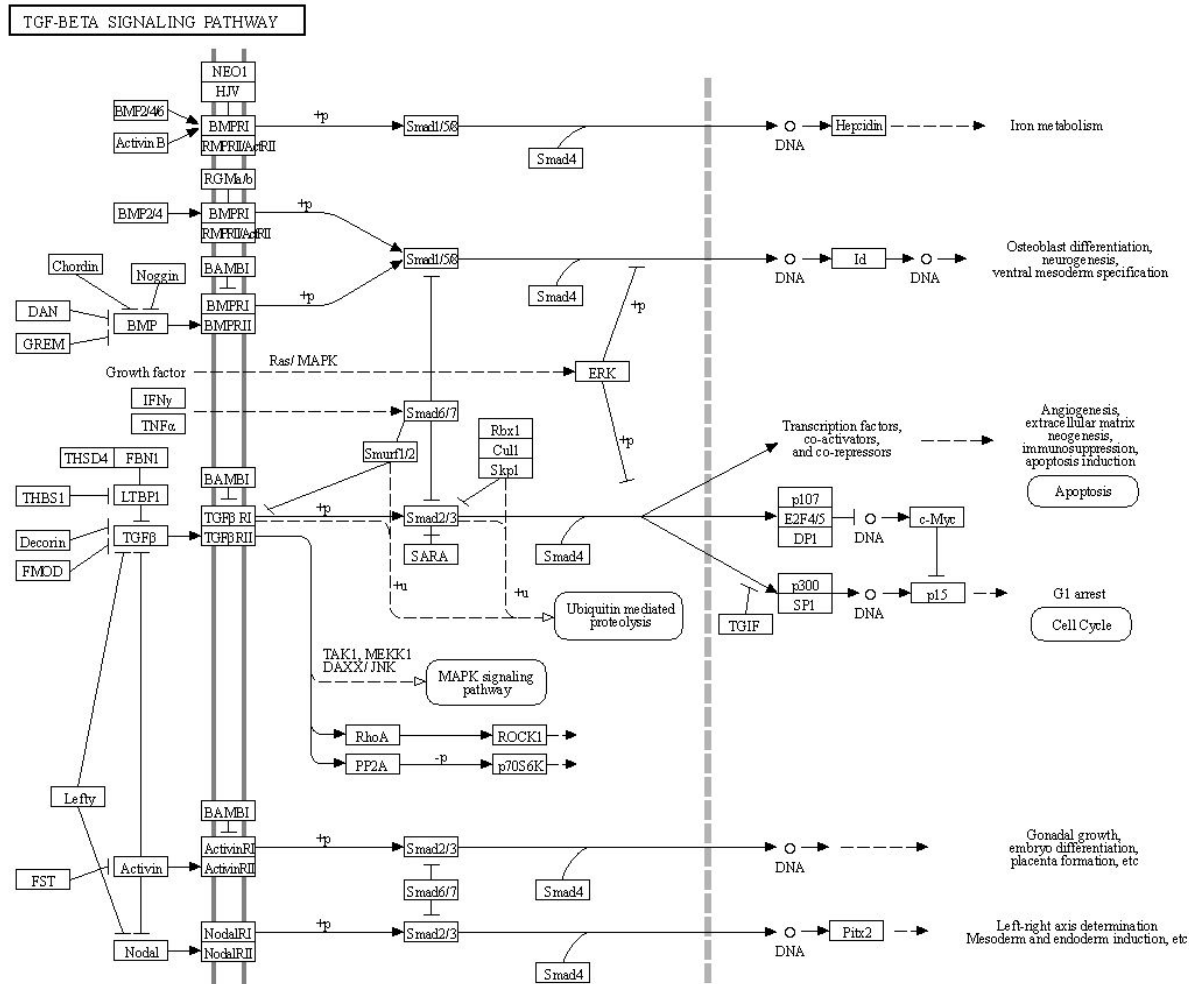


Roudnicky *et al.*, PNAS, 2020,  
<https://www.pnas.org/content/early/2020/08/04/1911532117>

# Mapping genes to biological pathways

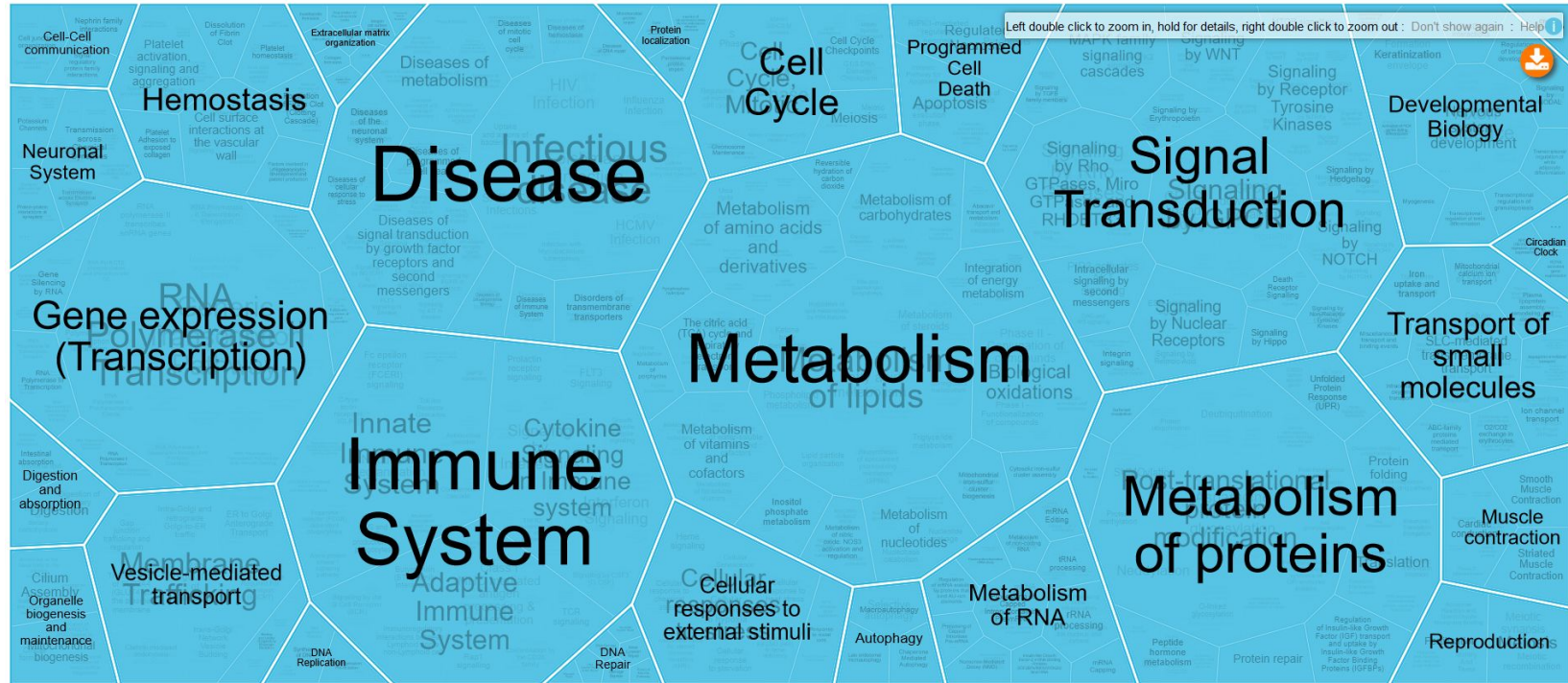
Option 1: [KEGG pathways](#), with the example of [TGF- \$\beta\$  signaling pathway](#).

[A RESTful API](#) is available for academic use, with clients in Python and R.





# Overview of pathways captured by Reactome



The Voronoi (Reacfoam) view of all pathways in Reactome



# Mapping genes to biological processes

- Gene Ontology
- UniProtKB keywords
- Example:  
TGFBFR2\_HUMAN  
(TGF-beta receptor type  
-2, P37173)

## Keywords

Molecular function	Kinase, Receptor, Serine/threonine-protein kinase, Transferase
Biological process	Apoptosis, Differentiation, Growth regulation
Ligand	ATP-binding, Magnesium, Manganese, Metal-binding, Nucleotide-binding

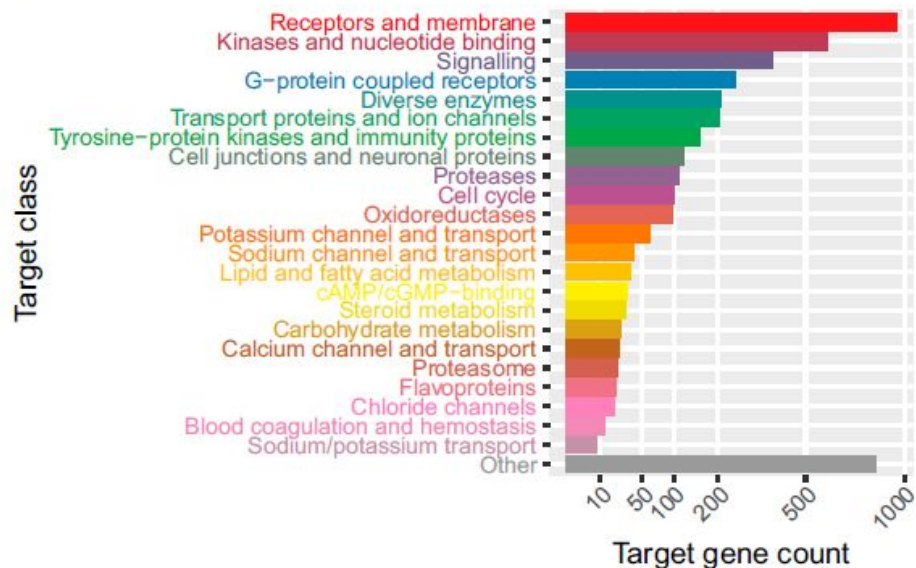


GO - Biological process

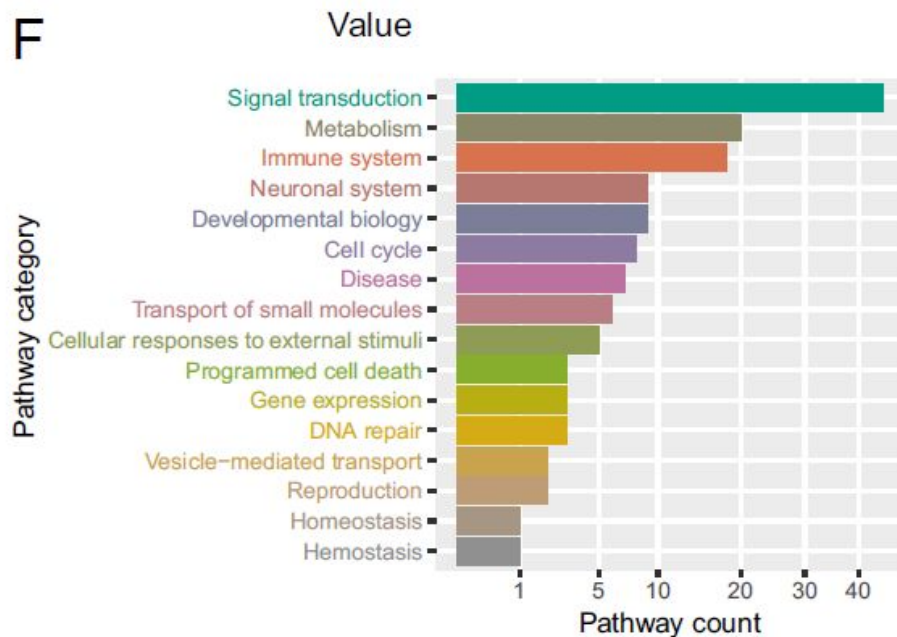
- activation of protein kinase activity Source: BHF-UCL
- aging Source: Ensembl
- animal organ regeneration Source: Ensembl
- apoptotic process Source: UniProtKB
- atrioventricular valve morphogenesis Source: BHF-UCL
- blood vessel development Source: BHF-UCL
- brain development Source: BHF-UCL

# SPARK covers the target space evenly with representative, potent, and specific compounds

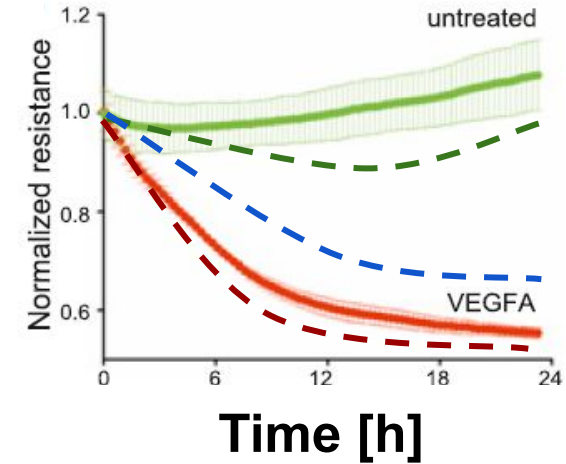
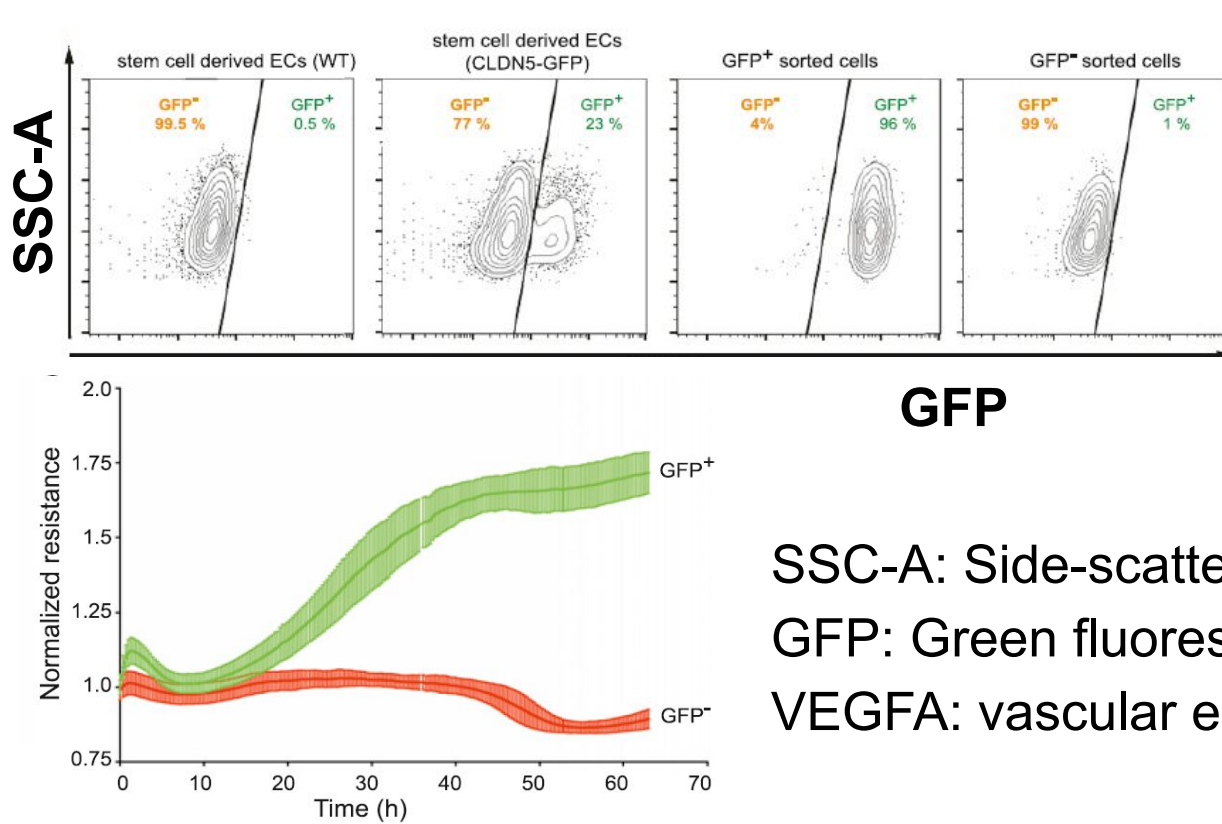
E



F

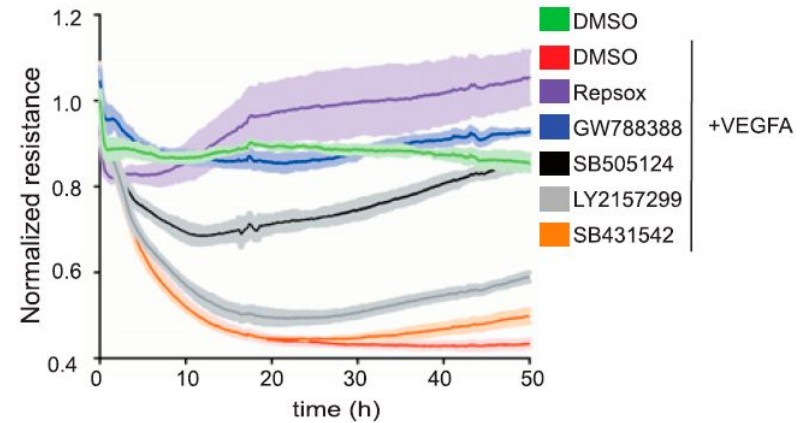
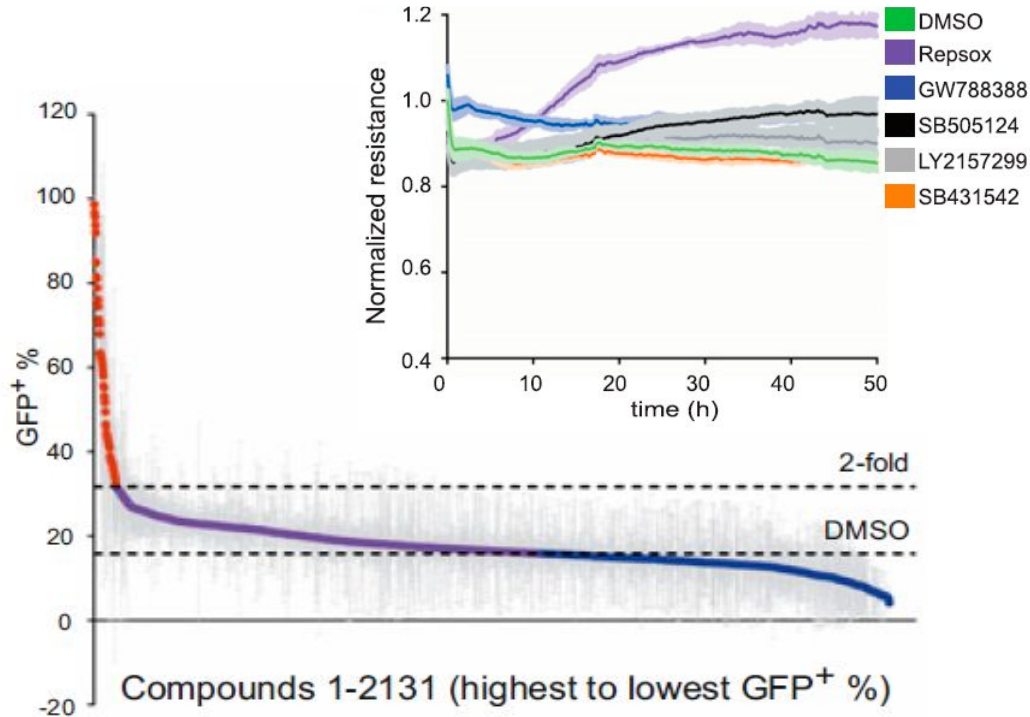


# Screening with stem-cell-derived endothelial cells with a reporter added by genome editing



SSC-A: Side-scatter area of flow cytometry;  
GFP: Green fluorescent protein;  
VEGFA: vascular endothelial growth factor A

# Compounds targeting the TGF- $\beta$ pathway such as RepSox modulates endothelial cells

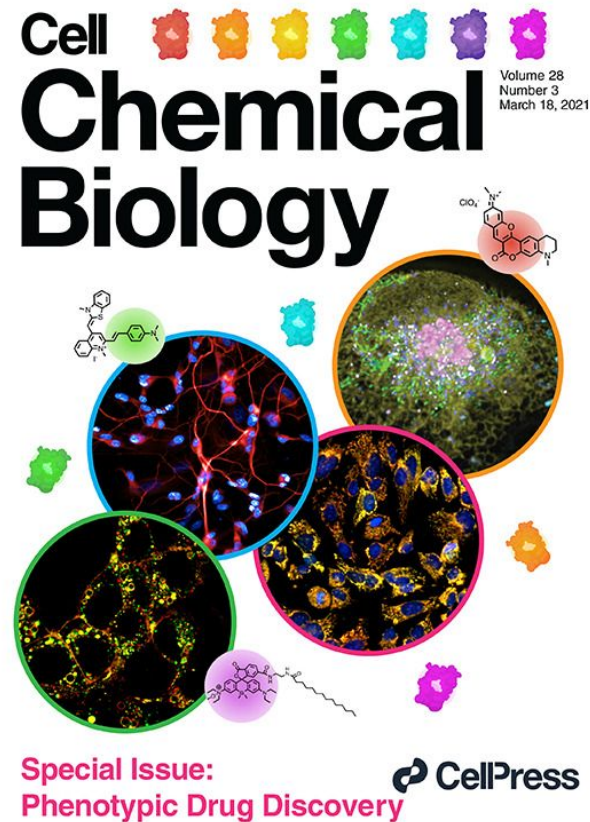


Further *in vitro* and *in vivo* experiments establish RepSox as a tool compound modulating retinopathy.



# Conclusions about chemogenomic library

- Phenotypic drug discovery can lead to first-in-class drugs with novel mechanisms;
- Unsupervised machine learning and data modelling contribute to build chemogenomic libraries;
- We can link drug candidates via targets to biological pathways and processes.

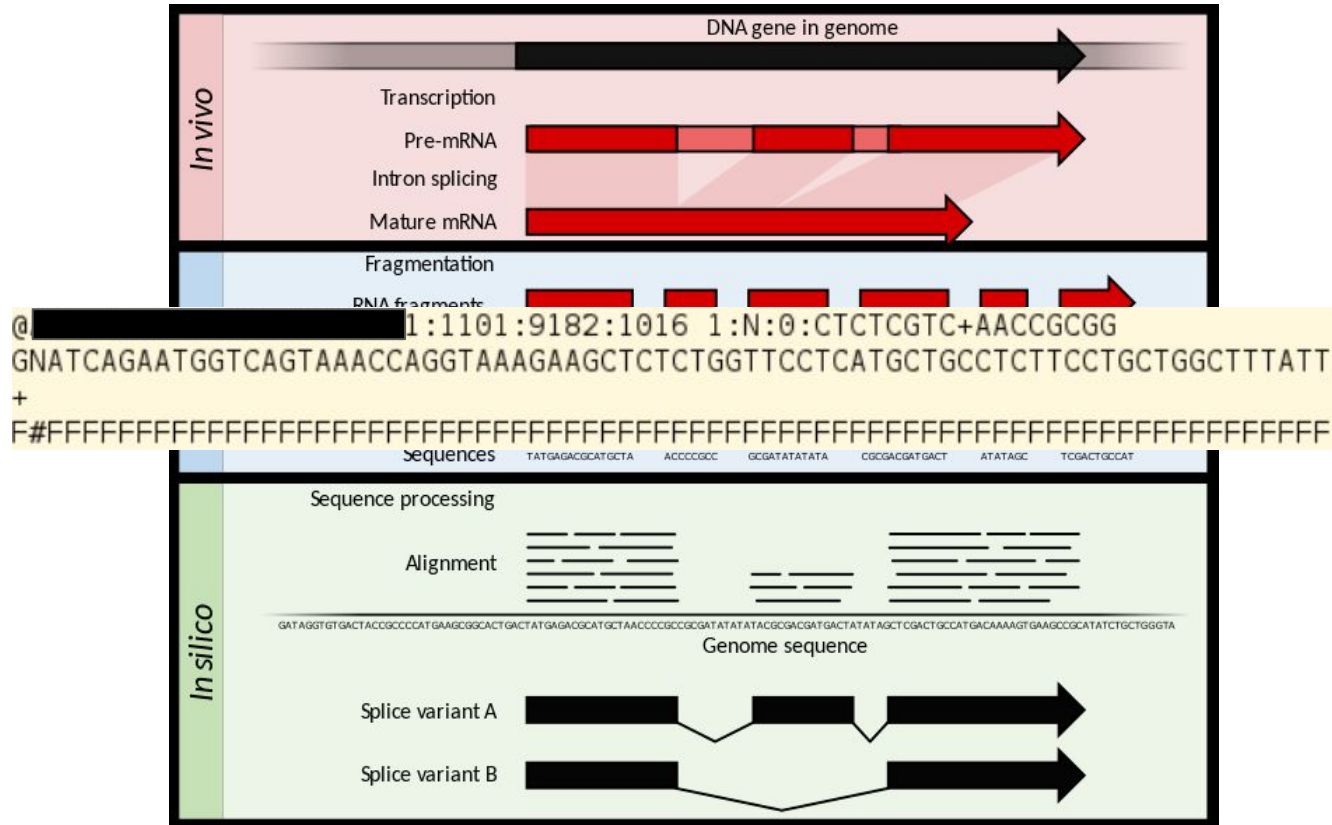


# Offline activities of Module II

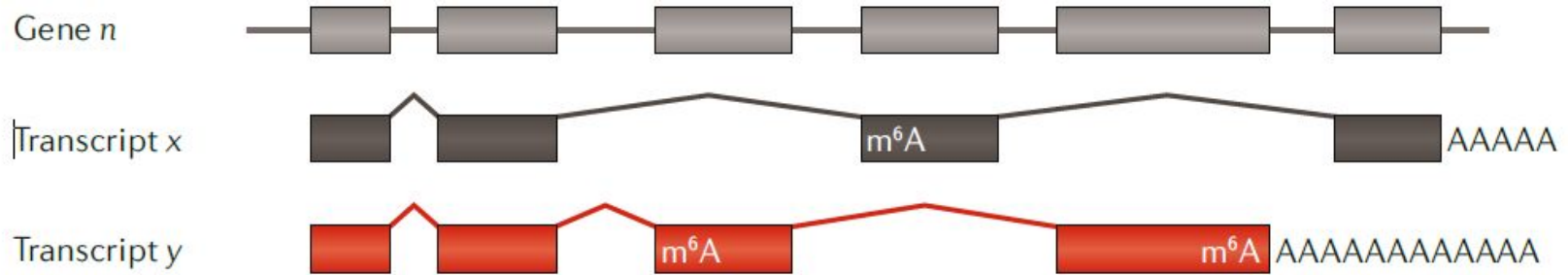
Please use your favourite programming language (shell scripts, python, R, for instance) and APIs (Application Programming Interfaces) of databases to perform following operations. Submit your code.

1. Retrieve all approved drugs from the ChEMBL database, sort them by approval year and name ([a Python example is here](#); documentations of the ChEMBL API can be found [here](#));
2. For each approved drug **since 2011** that you identified in step (1), retrieve a list of UniProt accession numbers, namely protein targets associated with the drug;
3. For each protein with a UniProt accession number that you identified in step (2), retrieve UniProt keywords associated with it. [You can use the UniProt API, documented here](#). [Python](#) and [R](#) clients are also available.

# Transcriptome profiling by RNA sequencing



# Transcriptome profiling by RNA sequencing



Ambiguous  
to exon



Unambiguous  
to exon



Ambiguous  
to isoform



Unambiguous  
to isoform

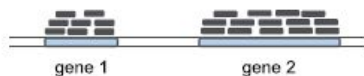


# Read Mapping

## Count collection

## Normalization by library size

## Differential Gene Expression Analysis



	sample A1	sample A2	sample B1	sample B2
gene 1	8	10	100	200
gene 2	14	15	15	40
gene 3	33	40	35	70
...	...	...	...	...
gene N	100	120	105	220

	sample A1	sample A2	sample B1	sample B2
gene 1	8	10	100	200
gene 2	14	15	115	40
gene 3	33	40	35	70
...	...	...	...	...
gene N	100	120	105	220

Tot. reads:  
5 millions

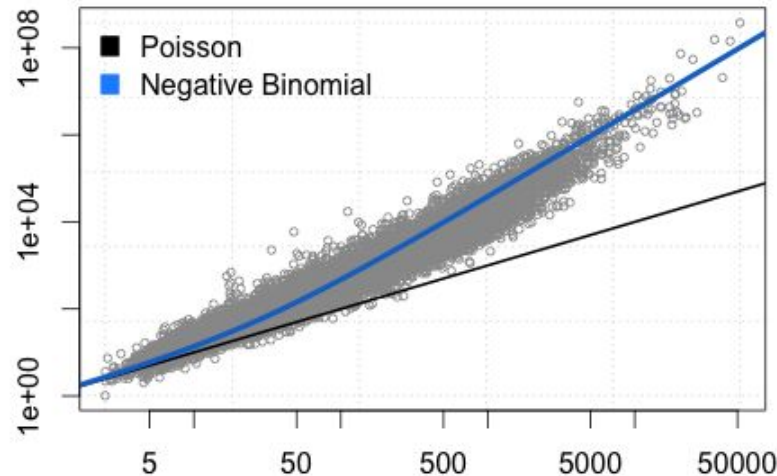
Tot. reads:  
10 millions

	sample A1	sample A2	sample B1	sample B2
gene 1	0.16	0.20	2.00	2.00
gene 2	0.28	0.30	0.30	0.40
gene 3	0.66	0.80	0.70	0.70
...	...	...	...	...
gene N	2.00	2.40	2.10	2.20

# Differential gene expression



Pooled gene-level variance (log10 scale)



Mean gene expression level (log10 scale)

Tools: *edgeR* and *DESeq2*

# Interpret differential gene expression data with gene-set enrichment analysis

Reactome pathways

Gene Ontology

UniProt Keywords

Literature

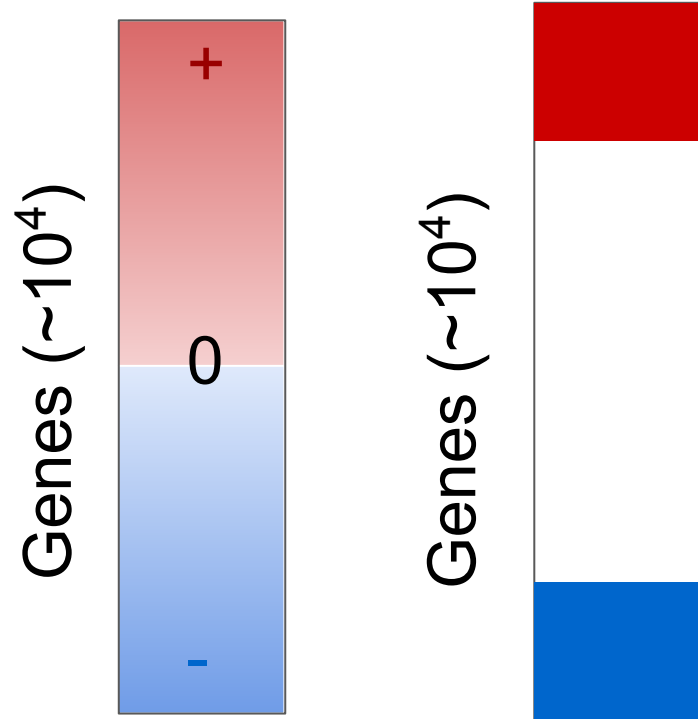
Gene (N~10 <sup>4</sup> )	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	...	G <sub>N-3</sub>	G <sub>N-2</sub>	G <sub>N-1</sub>	G <sub>N</sub>
Change (log2)	3.0	2.8	2.5	1.5	1.2	...	-0.8	-1.2	-1.5	-2.2

Differential gene expression results

Gene-set Enrichment Analysis Methods

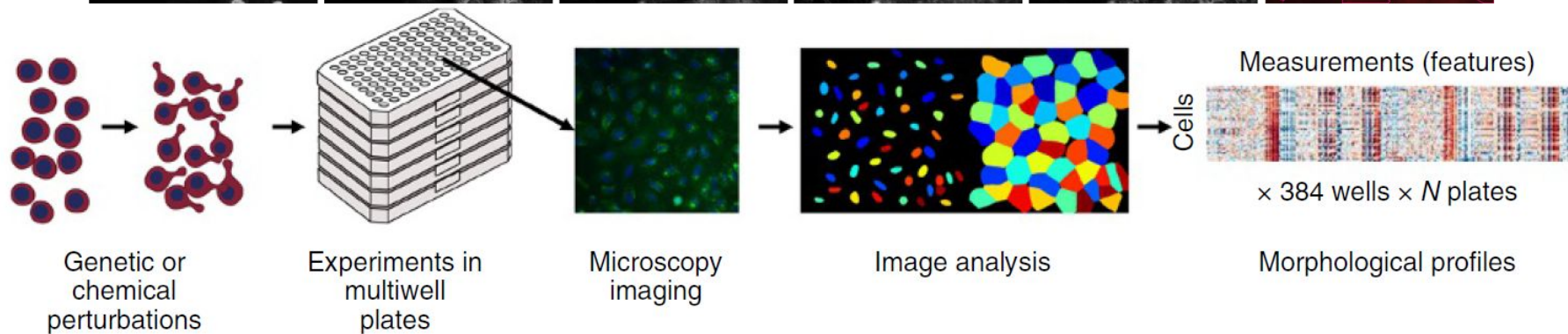
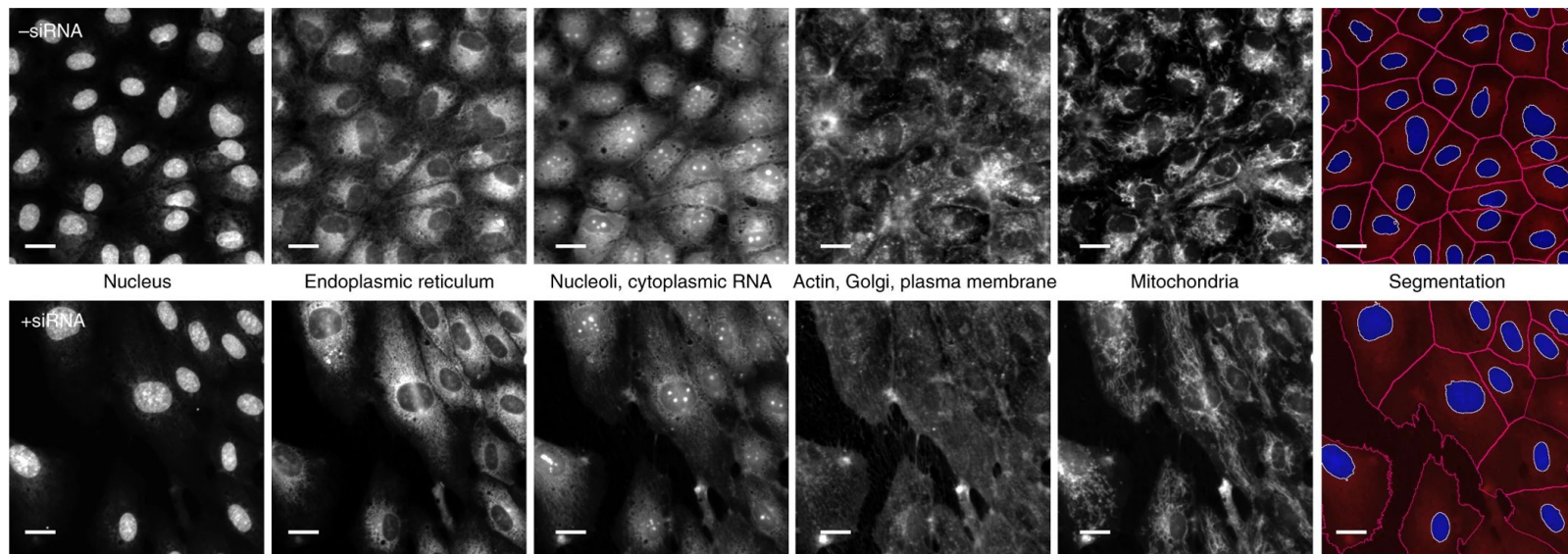
# Gene-set enrichment analysis with or without a hard threshold

An arbitrary set of genes  $G$



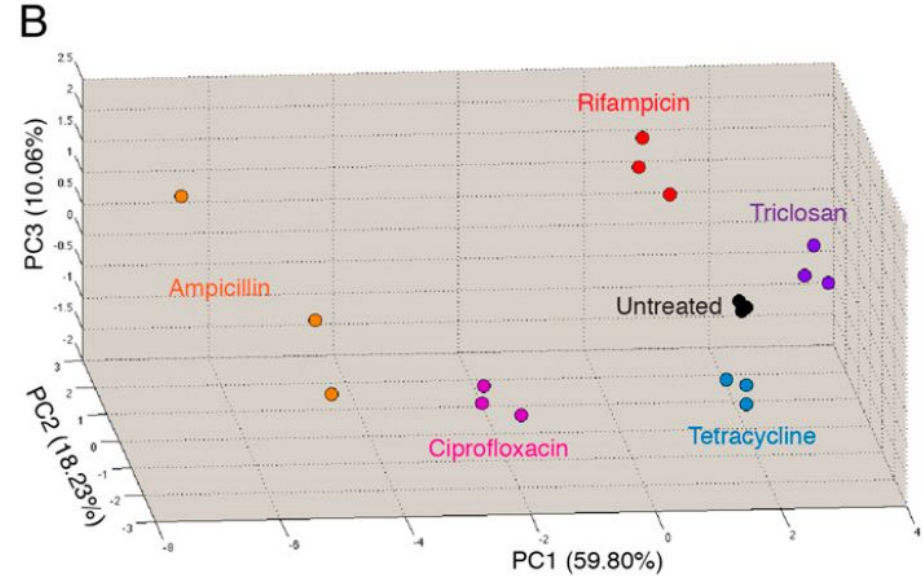
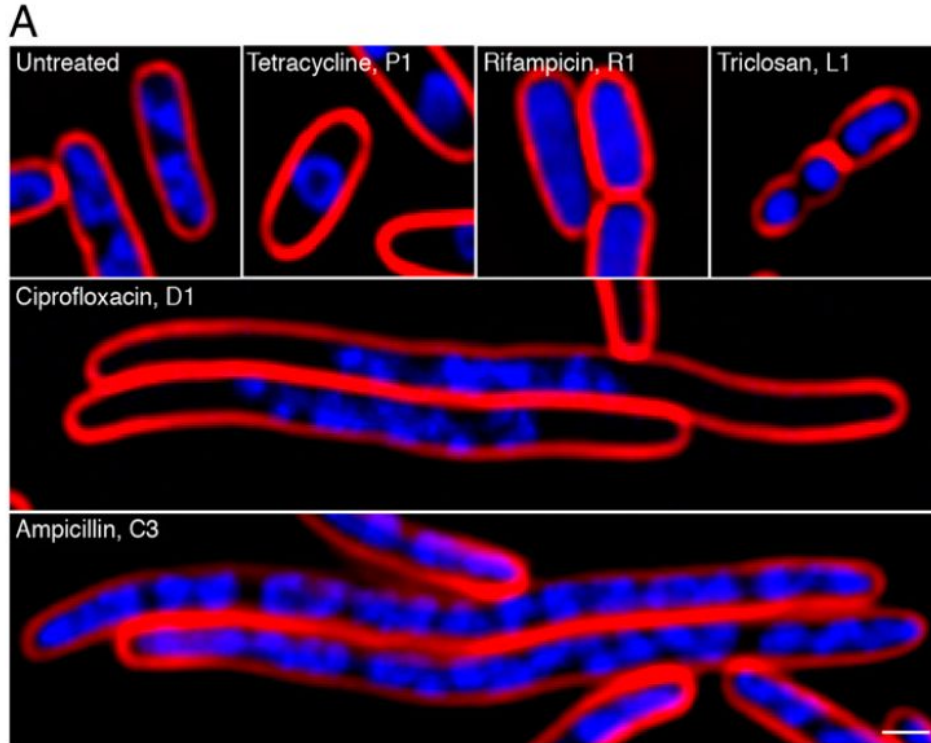


# Morphology as screening readout





# Cytological profiling for antibiotics discovery



**P:** Protein translation inhibitors

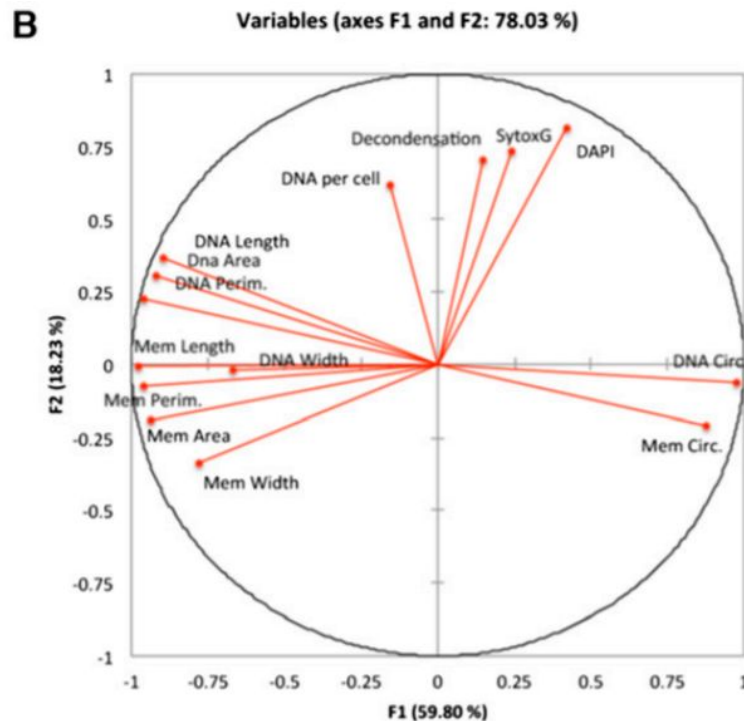
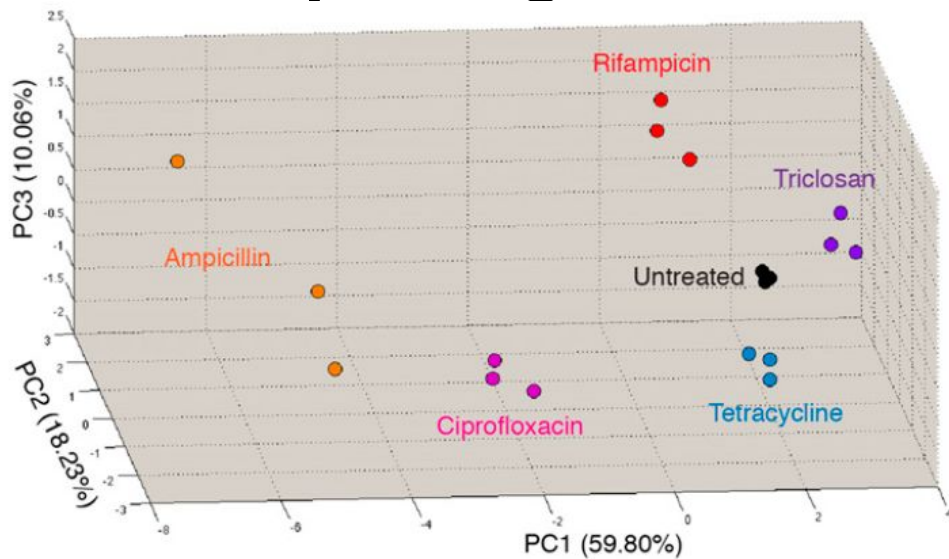
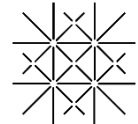
**R:** RNA transcription inhibitors

**D:** DNA replication inhibitors

**L:** Lipid biosynthesis inhibitors

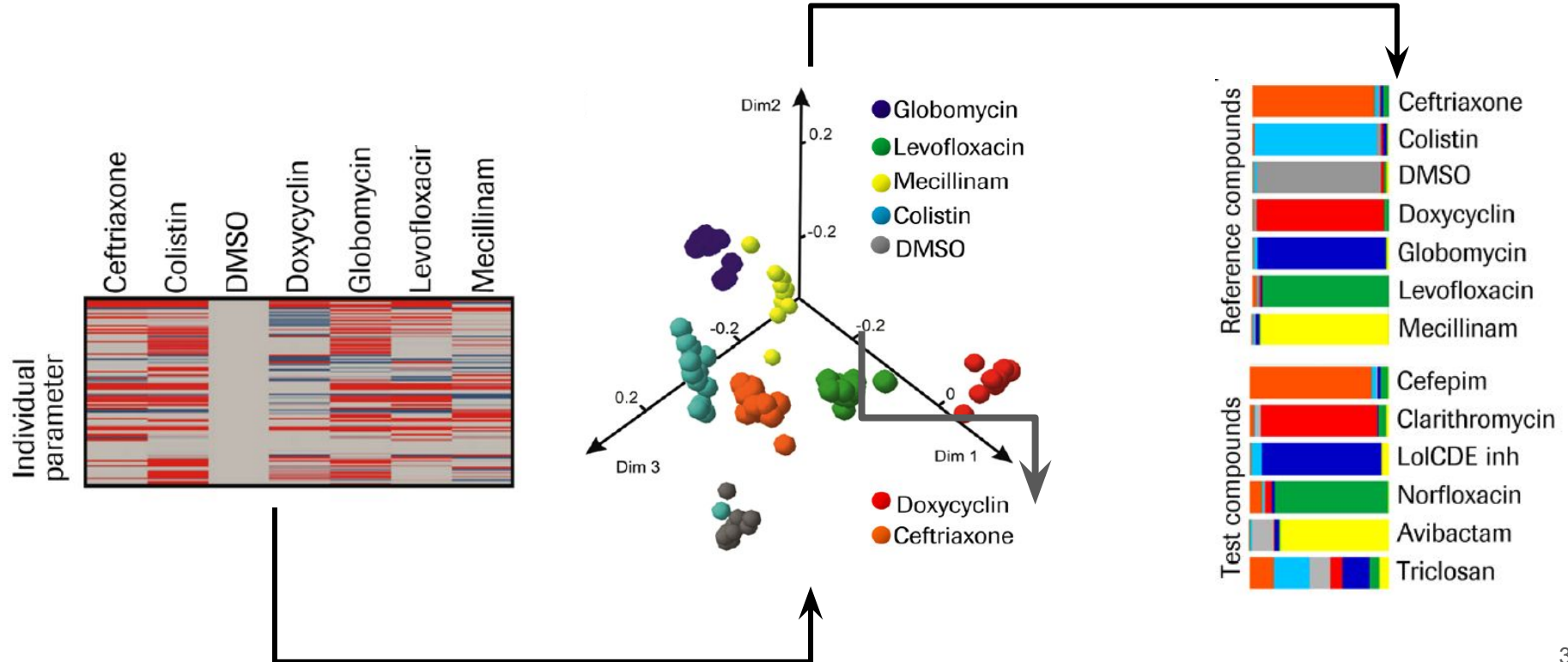
**C:** Cell-wall synthesis inhibitors (peptidoglycan)

# Principal components are linear combination of morphological features

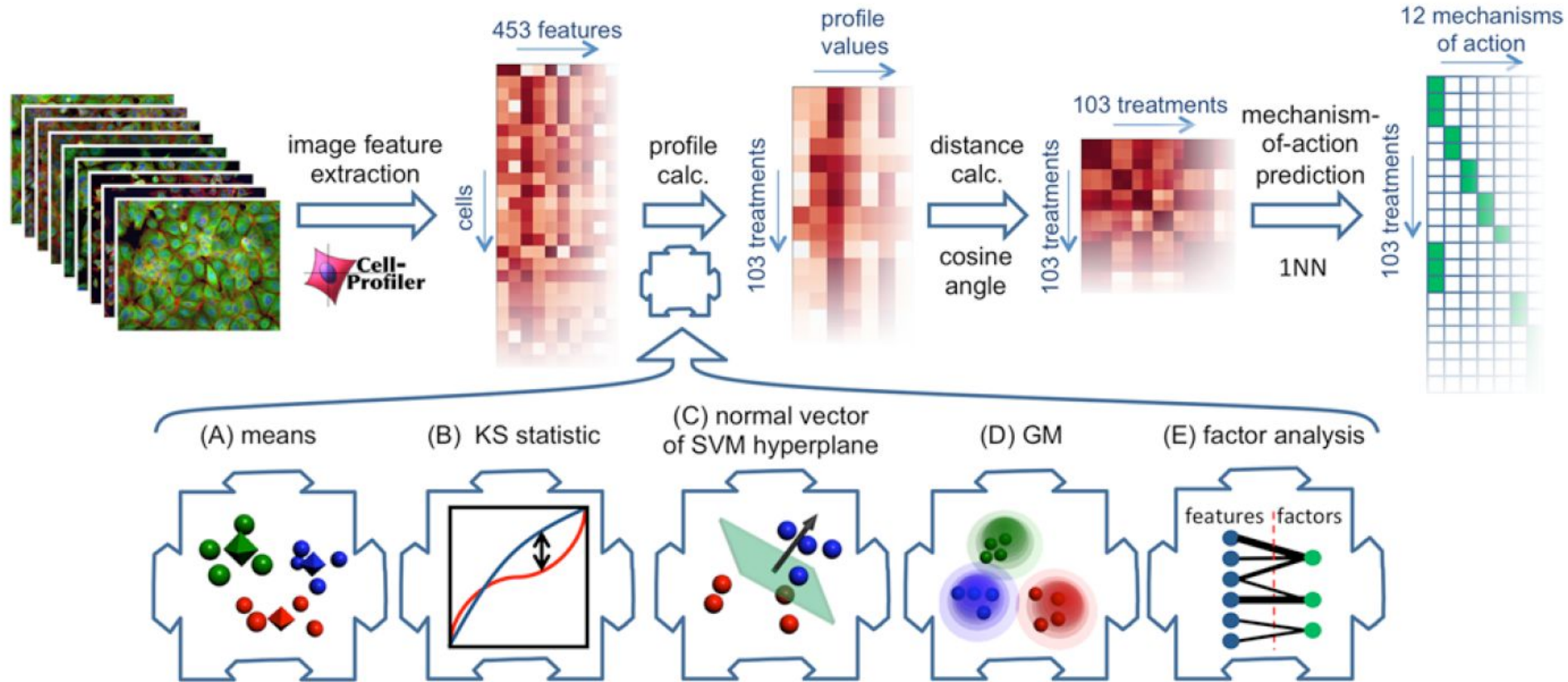


Membrane area, $\mu\text{m}^2$	DNA area, $\mu\text{m}^2$	Membrane perimeter, $\mu\text{m}$	DNA perimeter, $\mu\text{m}$	Membrane length, $\mu\text{m}$	DNA length, $\mu\text{m}$	No. of nucleoids per cell
Membrane width, $\mu\text{m}$	DNA width, $\mu\text{m}$	Membrane circularity	DNA circularity	SytoxG intensity	DAPI intensity	Decondensation

# Morphology classifies compounds by MoA



# Comparison of computational methods



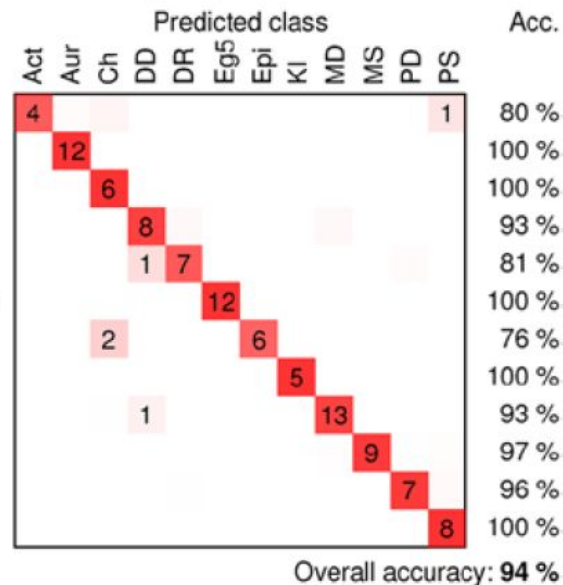
# Do the benchmark and use Occam's Razor

**Table 1.** Accuracies for classifying compound treatments into mechanisms of action.

Method	Accuracy, %
Means	83
KS statistic	83
Normal vector to support-vector machine hyperplane	81
With recursive feature elimination	64
Distribution over Gaussian mixture components	83
Factor analysis + means	94

True mechanistic class

Actin disruptors	Act
Aurora kinase inhibitors	Aur
Cholesterol-lowering	Ch
DNA damage	DD
DNA replication	DR
Eg5 inhibitors	Eg5
Epithelial	Epi
Kinase inhibitors	KI
Microtubule destabilizers	MD
Microtubule stabilizers	MS
Protein degradation	PD
Protein synthesis	PS

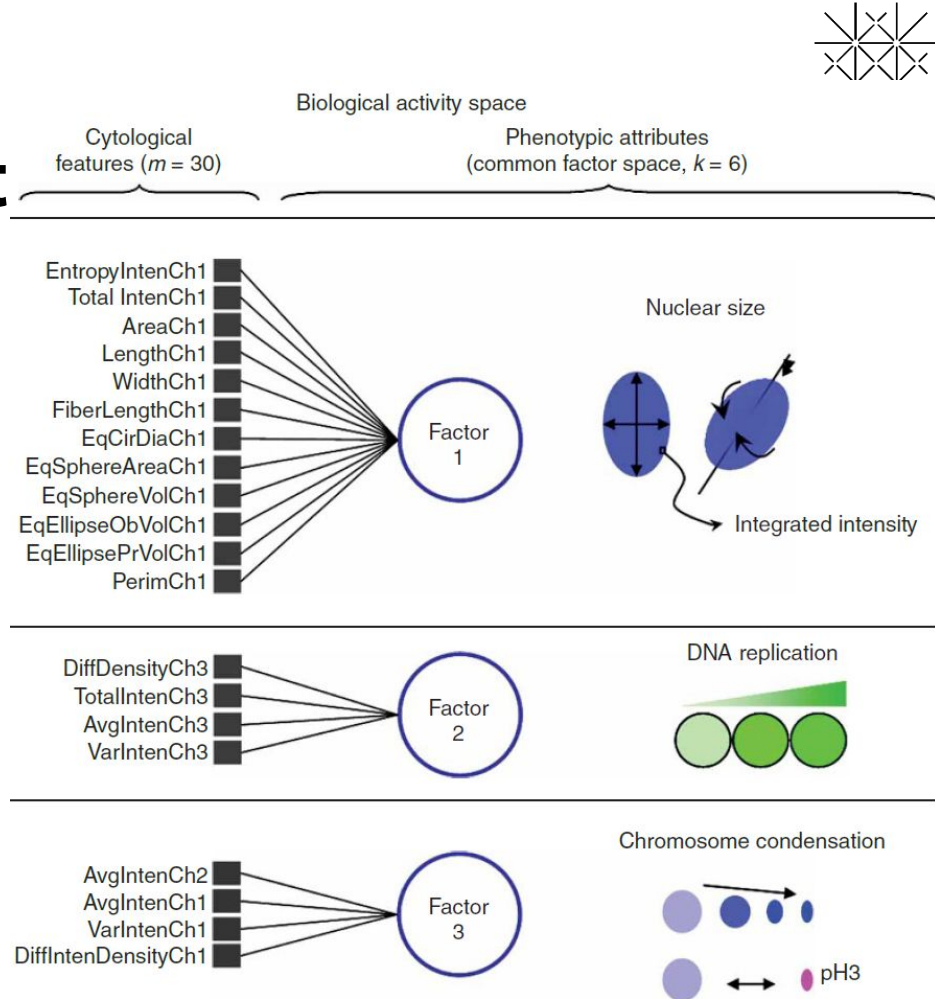




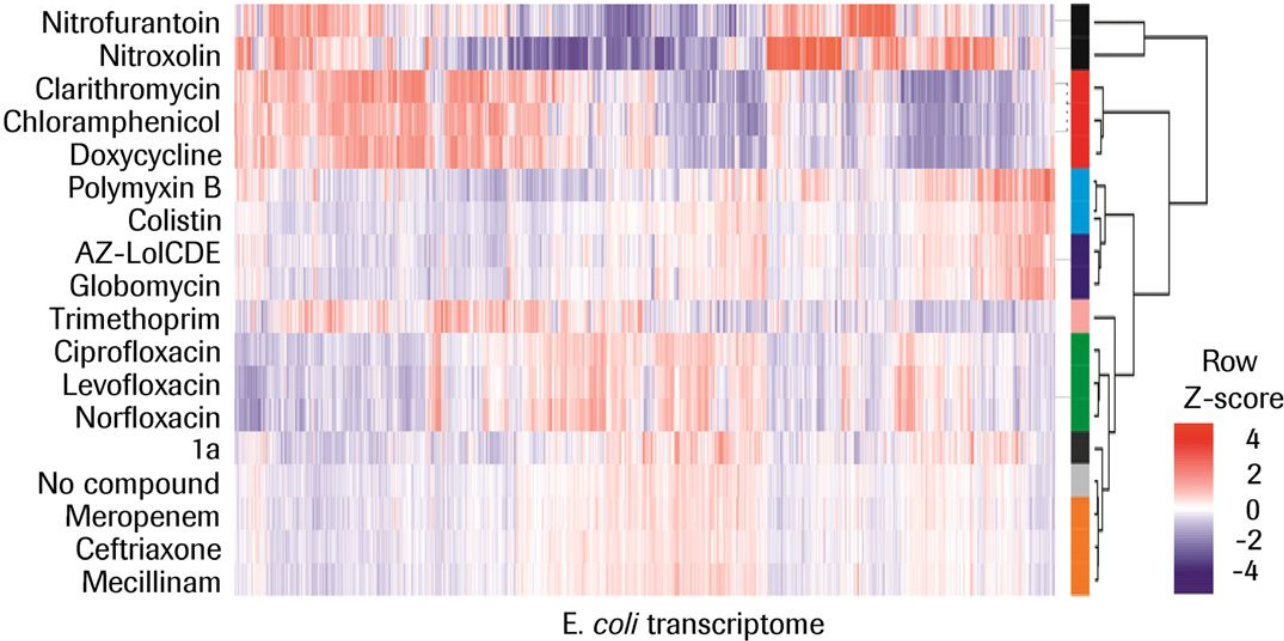
# A possible explanation for the success of latent variable models

$$\begin{matrix} \text{Cells} \\ \begin{pmatrix} x_{11} & \dots & x_{1m} \\ \vdots & \ddots & \vdots \\ x_{n1} & \dots & x_{nm} \end{pmatrix} \\ \text{Cytological features} \end{matrix} = X_{nm} = \underbrace{\sum_{i=1}^k L_{ni} F_{im}}_{k\text{-factor space}} + \varepsilon_{nm}$$

## A common latent factor model



# Morphology and gene expression used jointly

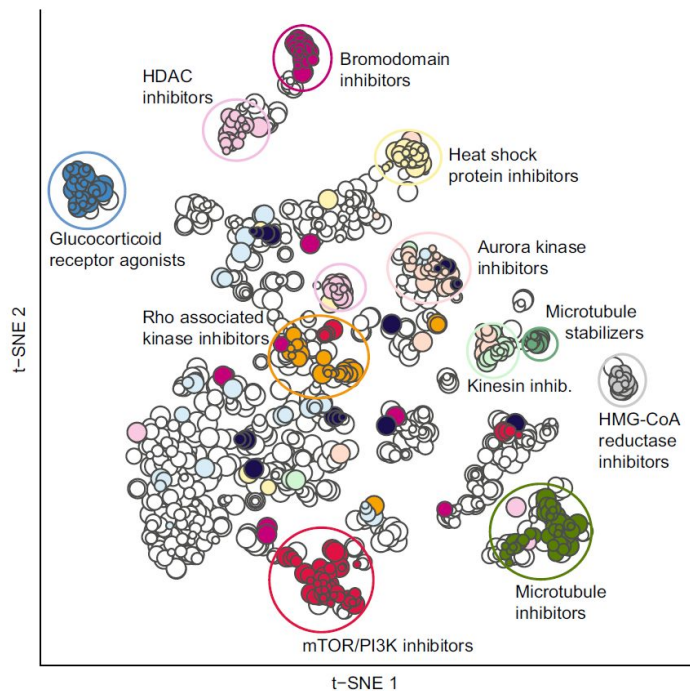


Gene-set  
enrichment  
analysis

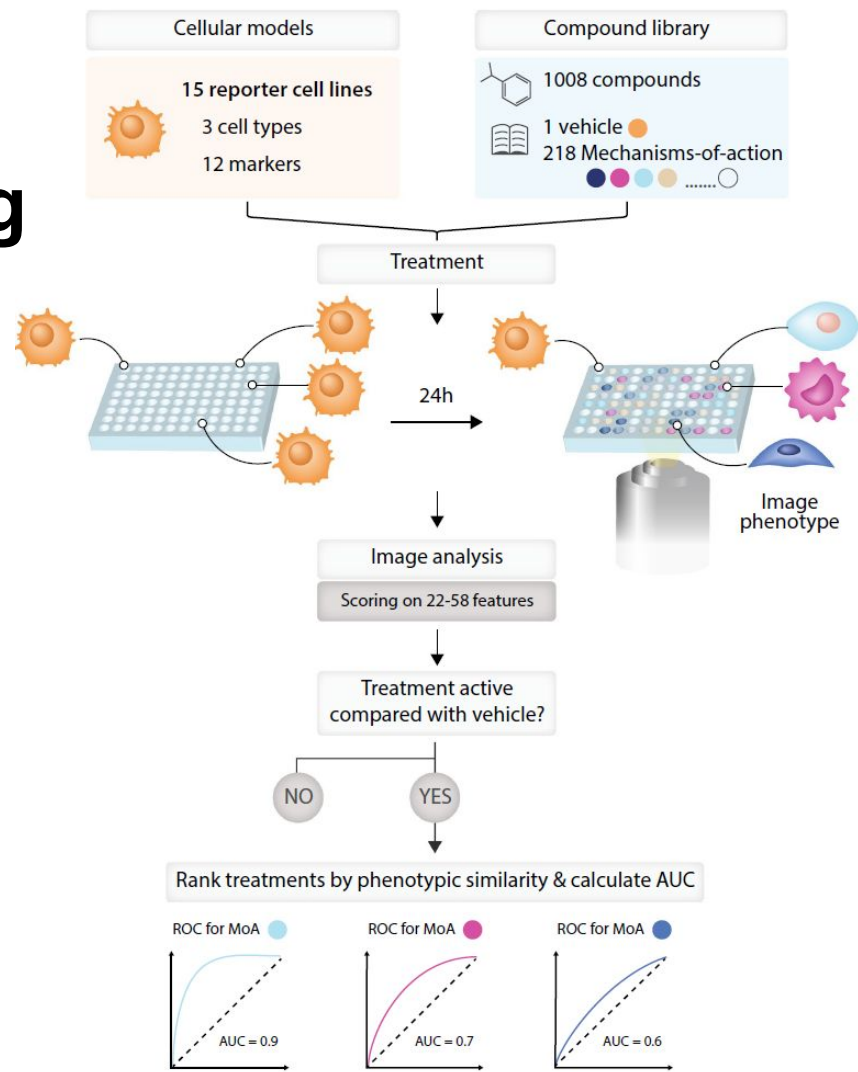
Reporter  
assays

Pathway-  
Phenotype  
associations

# A multi-cell-type, 1008-compound screening by Cox *et al.* (2020)



- 0.3  $\mu$ M
- 1  $\mu$ M
- 3  $\mu$ M
- 9  $\mu$ M
- ABL1 inhibitor
- Aurora kinase inhibitor
- Bromodomain inhibitor
- Glucocorticoid receptor agonist
- HDAC inhibitor
- Heat shock protein inhibitor
- HMG-CoA reductase inhibitor
- Kinesin inhibitor
- Microtubule inhibitor
- Microtubule stabilizer
- mTOR and/or PI3K inhibitor
- Rho associated kinase inhibitor
- VEGFR family inhibitor
- (Other)





# Conclusions

- Gene expression and image-based profiling can be used individually or jointly for phenotypic screening;
- Integration of biological knowledge, high-throughput data, and statistical modelling empowers phenotypic drug discovery.

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10. Ha, Jaeyoung, Hankum Park, Jongmin Park, and Seung Bum Park. 2021. "Recent Advances in Identifying Protein Targets in Drug Discovery." *Cell Chemical Biology* 28 (3): 394–423. <https://doi.org/10.1016/j.chembiol.2020.12.001>.

# References (continued)



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